Asahara et al. relate the isolation of "putative endothelial cell (EC) progenitors or angioblasts from human peripheral blood" (abstract). It is disclosed that the cells were isolated by contacting human peripheral blood with magnetic beads coated with anti-CD34 or anti-Flk-1 antibodies (page 464). The resulting isolated cells ("MB^{CD34+} cells") were plated on tissue culture plastic or collagen, where "a limited number" of attached cells ("AT^{CD34+} cells") became spindle shaped, and proliferated for 4 weeks (page 964). It is also disclosed that a subset of MB^{CD34+} cells plated on fibronectin attached "promptly" and became spindle shaped within 3 days (page 904). The medium employed for cell culture experiments is described as M-199 with 20% FBS and bovine brain extract (footnote 14).

It is also disclosed that FACS analysis of AT^{CD34+} cells that were cultured on fibronectin for 7 days showed that those cells expressed CD34, CD31, Flk-1, Tie-2 and E selectin, all "markers of the EC lineage" (page 965). Asahara et al. relate that the injection of Dil-labeled MB^{CD34+} cells into the tail vein of mice with unilateral hind limb ischemia resulted in the integration of labeled cells in the neovascularized ischemic hind limb (page 965).

Claim 44 is directed to a population of expanded endothelial cells obtained by culturing, in contact with a collagen I-coated surface, buffy coat cells which are obtained from peripheral mammalian blood, in the presence of a cell culture medium containing an effective amount of VEGF, and which medium is free of bovine brain extract. As disclosed in the specification, the cells in the expanded endothelial cell population have the typical "cobblestone" morphology of endothelial cells (page 6, lines 26-27 and page 18, lines 3-5). In contrast, the cells obtained by Asahara et al. were "spindle shaped". Therefore, Asahara et al. do not anticipate Applicant's invention.

Levine et al. describe a method of culturing endothelial cells from solid tissue, i.e., from human blood vessels, such as human umbilical vein endothelial cells. The method employs a gelatin matrix supplemented with endothelial cell growth factor and heparin and/or a dextran sulfate (column 2, lines 3-14 and column 3, line 64-column 4, line 27). The resulting cultured cells were characterized as endothelial according to morphological and functional criteria (expression of "Factor VIII-related antigen") and production of angiotension-converting enzyme (column 4, lines 53-57). However, Factor VIII-related antigen is not an endothelial cell-specific

marker as it is also found on megakaryocytes. Moreover, Levine et al. provide no evidence that the cultured cells include an expanded population of endothelial cells, i.e., endothelial cells which have multiplied. Thus, Levine et al. do not anticipate Applicant's invention.

Therefore, withdrawal of the § 102(b) rejections is respectfully requested.

The 35 U.S.C. § 103 Rejections

The Examiner rejected claims 1-2, 5-6, 8-9, and 44 under 35 U.S.C. § 103(a) as being unpatentable over Dzau et al. (U.S. Patent No. 6,352,555) in view of Asahara et al. The Examiner also rejected claims 1 and 3 under 35 U.S.C. § 103(a) as being unpatentable over Dzau et al. in view of Asahara et al. and further in view of Levine et al. The Examiner further rejected claims 1, 4-5 and 7 under 35 U.S.C. § 103(a) as being unpatentable over Dzau et al. in view of Asahara et al. and further in view of Gupta et al. (Exp. Cell Res., 230:244 (1997)). In addition, the Examiner rejected claims 1 and 13-14 under 35 U.S.C. § 103(a) as being unpatentable over Dzau et al. in view of Asahara et al. and further in view of Solovey et al. (NEJM, 337:1584 (1997)). Finally, the Examiner rejected claims 1 and 10-12 under 35 U.S.C. § 103(a) as being unpatentable over Dzau et al. in view of Asahara et al. and further in view of Dementriou et al. (U.S. Patent No. 6,140,123). These rejections are respectfully traversed.

The present application claims the benefit of the filing date of U.S. application Serial No. 60/109,687, filed on November 24, 1998. The Dzau et al. patent issued from U.S. application Serial No. 09/349,344, filed July 8, 1999, which claims the benefit of the filing date of U.S. application Serial No. 60/092,358, filed on July 10, 1998.

The Dzau et al. patent generally relates that a prosthesis composed of a porous tube, a portion of which has a certain diameter, can be contacted with cells and then exposed to a pressure differential to retain cells in the pores on the inner surface of the prosthesis (abstract). It is also disclosed that endothelial cells may be obtained by culturing a sample of mononuclear cells obtained from blood, e.g., the buffy coat, without further cell separation on a cell adhesive polymer-coated solid support, e.g., a fibronectin- or collagen-coated tissue culture plate, in the presence of endothelial growth factors, e.g., VEGF, bFGF and IGF, so as to result in an

endothelial cell culture having at least 90% endothelial cells or progenitors thereof (column 3, lines 6-22, column 4, lines 32-36 and column 7, lines 13-61).

Nevertheless the Dzau et al. '358 application does not disclose or suggest a method for obtaining endothelial cells from blood, i.e., the sections corresponding to column 3, lines 6-22, column 4, lines 25-36, and column 7, lines 13-61 in the Dzau et al. patent are not present in the '358 application. Therefore, the disclosure in the Dzau et al. patent relating to obtaining endothelial cells from blood has an effective date of July 8, 1999 and so is not prior art to the present claims.

As discussed above, Asahara et al. do not disclose or suggest a method which yields an expanded population of endothelial cells from buffy coat cells and Levine et al. do not describe or mention the use of buffy coat cells to prepare an expanded population of endothelial cells.

Gupta et al. describe a method to culture human dermal microvascular endothelial cells (HDMEC) in which cells are isolated by panning with EN4, an anti-endothelial cell monoclonal antibody, then stimulated with VEGF, a method which is intended to reduce fibroblast contamination of the resulting isolated population (page 244). It is disclosed that HDMEC were obtained from newborn human foreskin and, after trypsinization, the cells were cultured for three days prior to exposure of the cells to EN4 coated tissue culture plates (page 245). It is further disclosed that the panned cells, which were trypsinized and then cultured in gel-coated flasks, grew in circular colonies and exhibited cobblestone morphology (page 247). Gupta et al. do not mention buffy coat cells as a source for endothelial cells.

Solovey et al. disclose that immunohistochemical examination of buffy coat smears with P1H12, a monoclonal antibody which specifically reacts with endothelial cells, was used to enumerate circulating endothelial cells in normal blood donors, patients with sickle cell anemia and patients with non-hemoglobin S anemia (abstract and page 1585). For qualitative studies, circulating endothelial cells were isolated by two different methods: one employed P1H12 and the other employed P1H12-coated beads (page 1585). It is also disclosed that to determine whether P1H12-positive cells from the blood of patients with sickle cell anemia "remain alive", a population of P1H12-positive cells was contacted with an intracellularly-retained fluorescent dye and then cultured with primary microvascular endothelial cells for up to 28 days (citing to Gupta

et al.) (page 1586). Solovey et al. do not teach or suggest that circulating endothelial cells expanded in culture. Further, Solovey et al. fail to disclose or suggest a method in which endothelial cells are isolated from any source in the absence of antibody.

Dementriou et al. disclose a method for preconditioning and cryopresentation of cells harvested from a donor (abstract). Dementriou et al. do not disclose or mention endothelial cells or a method to expand such cells.

With respect to the rejection of claims 1-2, 5-6, 8-9, and 44 over Dzau et al. and Asahara et al.; claims 1 and 3 over Dzau et al., Asahara et al., and Levine et al.; claims 1, 4-5 and 7 over Dzau et al., Asahara et al. and Gupta et al.; claims 1 and 13-14 over Dzau et al., Asahara et al. and Solovey et al.; and claims 1 and 10-12 over Dzau et al., Asahara et al., and Dementriou et al., as discussed above, Dzau et al. is not available as prior art to the pending claims. Moreover, as none of Asahara et al., Levine et al., Gupta et al., Solovey et al., or Dementriou et al. disclose or suggest a method to expand endothelial cells from buffy coat cells, there is no combination of the available cited art which renders Applicant's invention obvious.

Therefore, withdrawal of the § 103(a) rejections is respectfully requested.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-373-6959) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

ROBERT P. HEBBEL ET AL.,

By their Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 373-6959

Date September 5, 2002 By

Janet E. Embretson
Reg. No. 39,665

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on this 5th day of September, 2002.

Dawn M. Poole
Name

Dawn M. Poole
Signature